

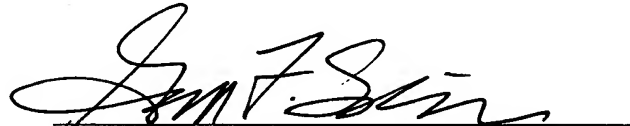
Express Mail No.: EV504788940US

Atty. Dkt. No. 15060-42

REMARKS

Please enter the foregoing preliminary amendment prior to examination of the present application. Applicant respectfully submits that this Amendment presents no new matter. Early passage to issue is requested.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Gordon F. Sieckmann", is written over a horizontal line.

Gordon F. Sieckmann, Reg. No. 28,667
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314-621-5070

IN THE DRAWINGS

Applicants respectfully request approval of the following drawing changes.

Figures 2, 10, 13, 21, 23, 26, 28, 31 and 33 are being amended to remove excessive text. Applicants hereby submit an “Annotated Copy” of Figures 2, 10, 13, 21, 23, 26, 28, 31 and 33 showing the requested changes in red permanent ink, and a “Replacement Sheet” incorporating the changes to Figures 2, 10, 13, 21, 23, 26, 28, 31 and 33.

No new matter has been added.



Title: CALCIUM INDEPENDENT PHOSPHOLIPASE A₂ γ
POLYNUCLEOTIDES AND POLYPEPTIDES AND METHODS
THEREFOR

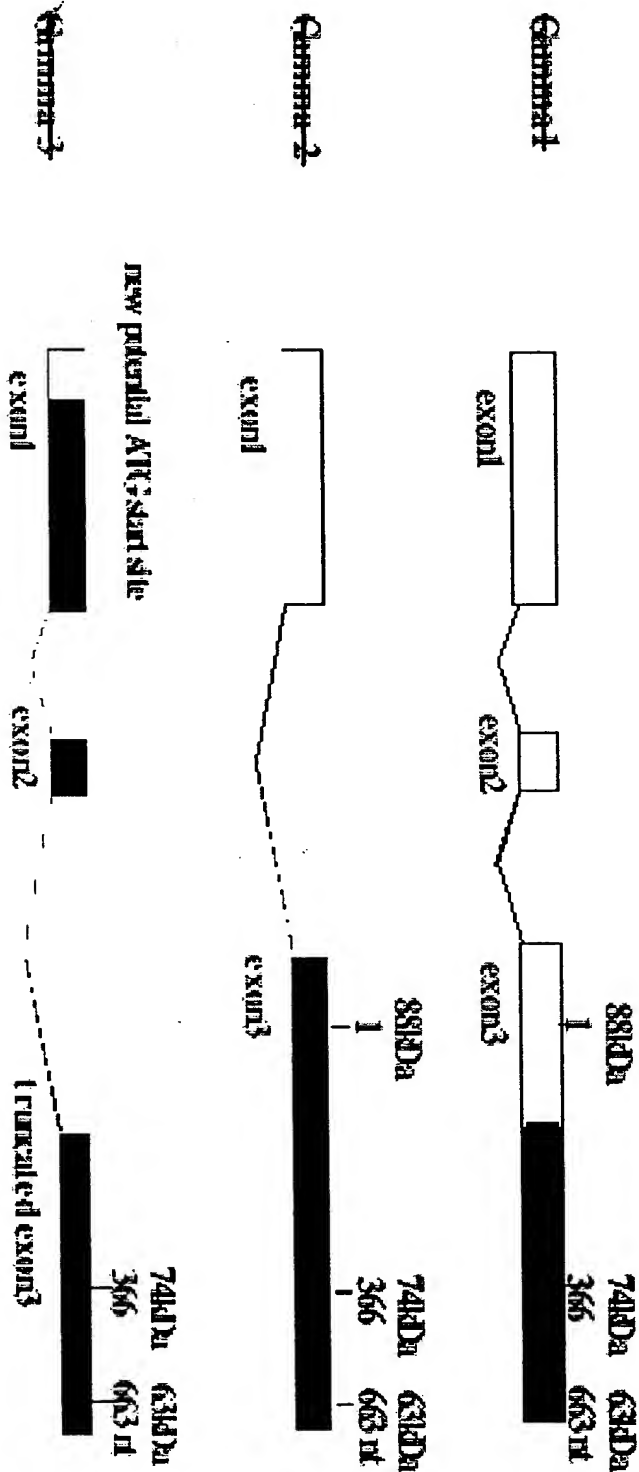
Inventor: Richard W. Gross et al.
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FIGURE 2

~~iPLA₂ γ Splice Variants~~



open boxes are noncoding regions
shaded regions are putative coding regions
stippled lines represent intron splicing

FIGURE 10

Potential Alternative Exon 5 Splice Variant of Human iPLA₂^γ

A. Reported Splice Sequence (gc/ag)

Exon 5 (SEQ ID NOS 43-44)	Intron 5	Exon 6 (SEQ ID NOS 45-46)	Source
...CAG CGA GAA AAG	gcaagt...ttgttag	ATT ATC GCA AGG GTG AGT	(Tamura et al)
Q R E K		I I A R V S	BBRC 272-320, 2000

B. Potential Splice Variant (gt/ag)

Exon 5 (SEQ ID NOS 47-48)	Intron 5	Exon 6	Source
..GAA AAG GCA AGT TGT TCA GT	gtgctt..tcgcaag	G GTG AGT	(Gross lab)
E K A S C S V		V S	BBRC 275-9937, 2000

The incidence of gc/ag splice variants like the one shown in "A" is 0.56%. The variant "A" has been reported in the literature, reported in GenBank, and cloned in our lab.

The splice variant gt/ag occurs with a frequency of 98.71% among genes. However, variant "B" iPLA₂^γ sequence has not been cloned.

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FIGURE 13

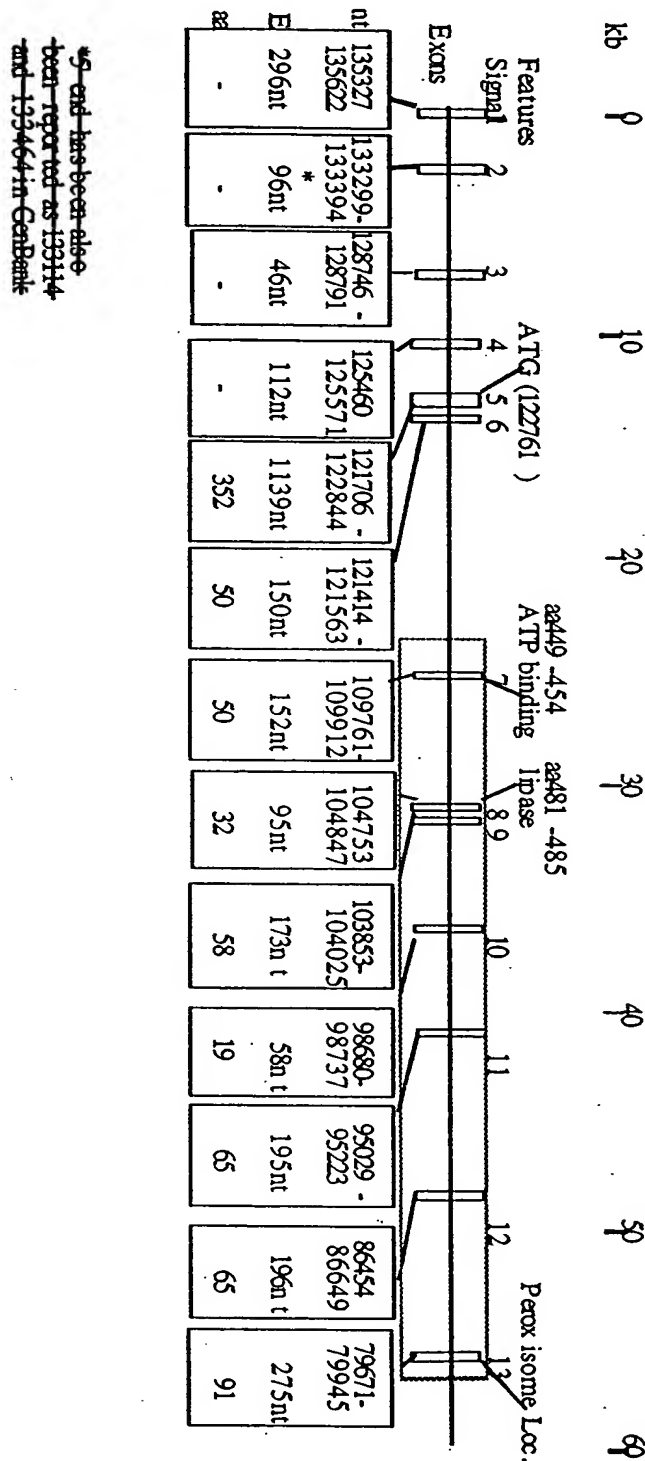
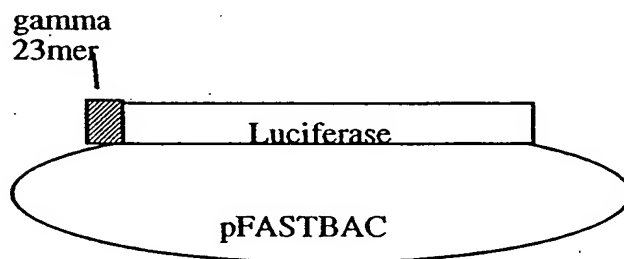


FIGURE 21

~~Additionally, iPLA₂γ sequences were inserted by ligation of 15-23mer annealed-phosphorylated oligonucleotide pairs 5' of full length luciferase coding sequence cloned into pFASTBAC via NotI/XbaI restrictions and then luciferase activity of recombinant protein produced in the Sf9 system was subsequently measured using the Luciferase Assay System of Promega.~~



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FIGURE 23. iPLA₂ Repressor Region

~~Phosphorylated oligo pairs~~
~~for repression of iPLA₂ in the luciferase expression system:~~

SEQ ID NO: 10	atgatttcacggttagctcaatttaagccaagtcaccaattttaagaaagatcggatagtgctggttaaaacagaaaaacatcaaca
SEQ ID NO: 32	tcgacctgatttcacggttagctcaatt
SEQ ID NO: 36	ggactaaagtgcaaatcgagtttaaccgg
SEQ ID NO: 33	
SEQ ID NO: 37	tcgactaagccaagtcaccaatttaa
	gattcgttcaagggtttaaatccgg
SEQ ID NO: 34	
SEQ ID NO: 38	tcgacgaaaagtatcgatagtgctgg
	gctttcatagcctatcacccgaccgg
SEQ ID NO: 35	
SEQ ID NO: 39	tcgacttaaaacagaaaaacatcaaca
	gaattttgtctttttgtagtttgcgg

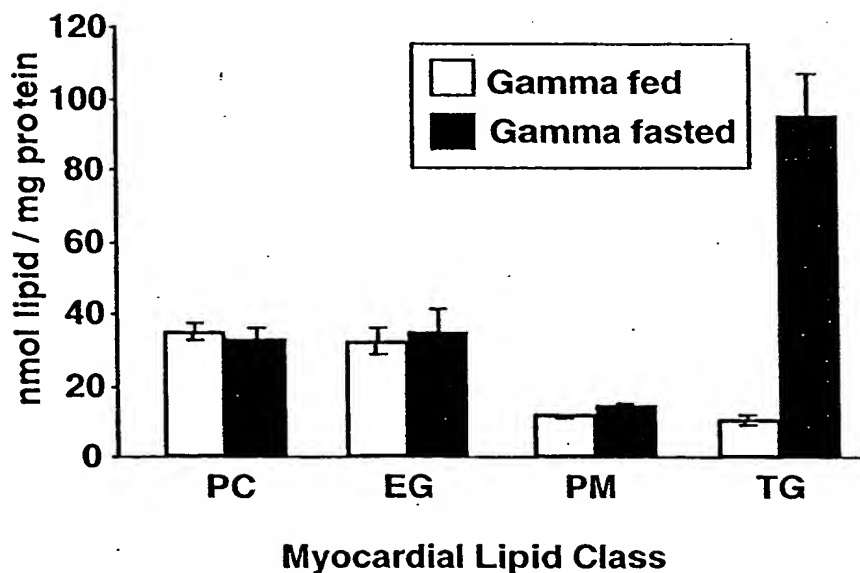
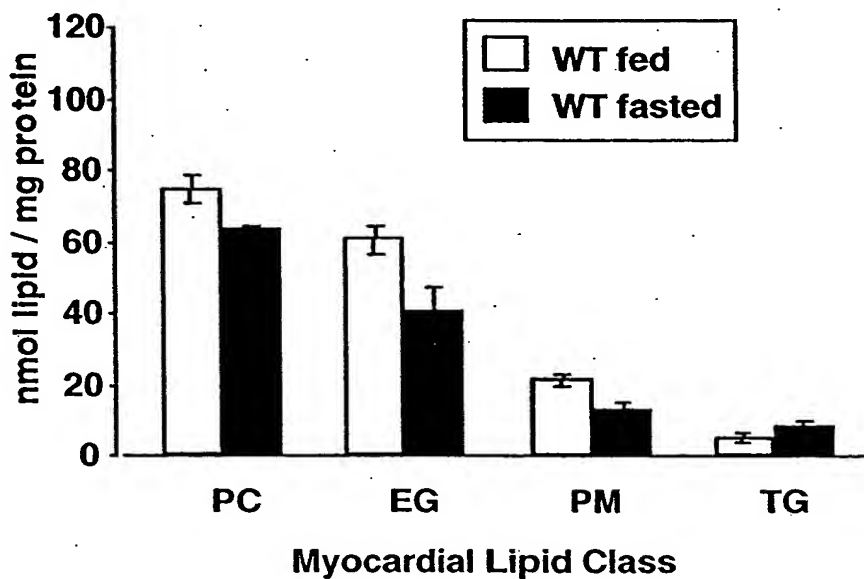
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Fig. 26 Myocardial TAG Content of Fasted WT vs iPLA₂ γ Transgenic Mice



PC = Phosphatidylcholine
EG = Ethanolamine Glycerophospholipids
PM = Plasmalogen
TG = Triacylglyceride

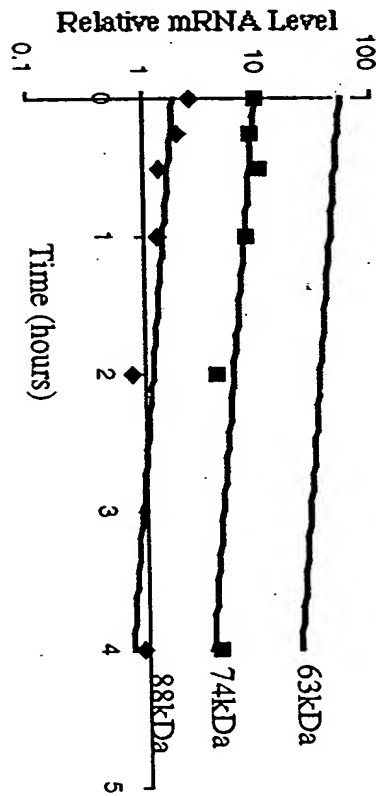
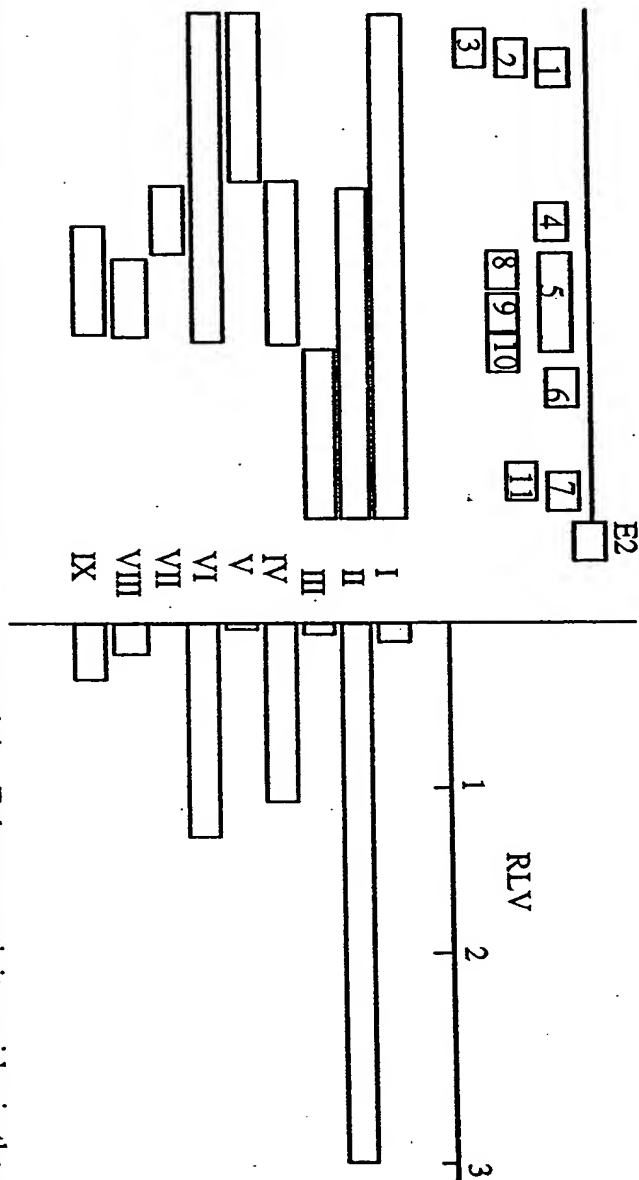


FIGURE 28. Quantitative PCR analysis of RNA stability of untreated iPLA₂ γ S9 Expression

FIGURE 31

FIGURE 33

Promoter Analysis of iPLA₂ γ Pre-exon 2



Conclusion: sequence upstream of exon 2 has promoter activity. Enhancer activity resides in the region 200-400nt upstream of exon 2 (fragment IV). This region contains a CACG-VNTR-like sequence as well as sequences that match consensus sites for Sp1 (8), GATA1 (9), p300 (4), and Gcrl (10). GC regions upstream (1) and downstream (7) of this positive promoter region commonly are negative regulatory elements. Truncated fragments (II and VI) each lacking a GC region have enhanced promoter activity while fragments (III and V) containing the GC regions but lacking region IV have minimal promoter activity. Presumably both GC regions are required for maximal inhibition. Region IV may have less than optimal promoter activity if positive promoter elements are immediately upstream or downstream of region IV.